

Effects of Infection on Oxygen Consumption and Core Temperature in Experimental Thermal Injury

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Oxygen consumption ($\dot{V}O_2$) and colonic temperature (T_c) were measured in groups of rats before and after 30% total body surface, full thickness burns. Some wounds were seeded with *Pseudomonas aeruginosa* or *Staphylococcus epidermidis*, and some seeded wounds were treated with Sulfamylon® or Silvaden®. Three groups became bacteremic (B) during the 2-3 week period of observation. At an ambient temperature (T_a) of 32 C, $\dot{V}O_2$ of the B groups rose from 0.83 ± 0.01 to 1.20 ± 0.01 ml/hr/g (mean \pm S.E., $p < 0.001$) versus 0.81 ± 0.01 to 0.99 ± 0.02 for nine nonbacteremic (NB) groups ($p < 0.001$). T_c increased only in the B groups—from 36.8 ± 0.1 to 37.7 ± 0.1 C ($p < 0.001$). In the second or third week postinjury, $\dot{V}O_2$ of the NB rats was reduced when T_a was increased to 34 C; T_c followed changes in T_a . Sulfamylon lowered $\dot{V}O_2$ of *P. aeruginosa* seeded, NB rats. The metabolic cost of wound contamination appeared to vary with bacterial strain. The metabolic effects of infection appear to be a continuum, beginning with a modest rise in $\dot{V}O_2$ and progressing to greater increases in $\dot{V}O_2$ and T_c with wound invasion and systemic infection.

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products are possible important metabolic stimuli in burned patients.

Metabolic responses of the burned patient are commonly separated into those developing before or after systemic infection. While this is an important clinical distinction, it fails to encompass the possible metabolic effects of bacteria prior to invasive infection. The purpose of this study was to determine to what extent bacterial contamination of the burn wound affects the energy metabolism and core temperature of nonbacteremic rats. We seeded burn wounds with different bacteria and treated some of these wounds with topical antimicrobial agents. The results indicate that bacterial growth in the burn wound increases oxygen consumption in nonbacteremic burned rats.

Materials and Methods

Animals

The animals selected for study were 3-7-month-old, male Sprague-Dawley rats (Holtzman, Madison, WI) weighing 400-600 g. They were housed in individual cages at an ambient temperature of 28-30 C and had access to food (Purina laboratory chow) and water throughout the study. A 12-hour light/dark cycle was maintained with lights coming on at 0600 daily.

Respiratory Gas and Core Temperature Measurements

Oxygen consumption was determined in groups of animals (13-30/group) using an open and closed respiration chamber.⁶ Chamber temperature was set at 30 C for uninjured animals and at 32 or 34 C for the burned animals.

POSTBURN HYPERMETABOLISM is a well-recognized clinical entity, but its etiology remains poorly understood. Thermoregulatory and nonthermoregulatory explanations have been offered, but neither is fully accepted.¹⁻³ Since the burn wound is never sterile during the hypermetabolic phase of injury⁴ and infection alone produces metabolic and neuroendocrine adjustments similar to those in thermal injury,⁵ bacteria and/or their

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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Average chamber temperature (mean of one wall temperature and air temperature for four different sites) varied ± 0.3 C. Relative humidity ranged from 40–50%.

The animals (each in its own cage) were left undisturbed in the chamber for at least 1 hour prior to gas exchange measurements. When the study began, large valves closed, making the chamber airtight. Chamber oxygen and carbon dioxide concentrations were determined at 15-minute intervals by mass spectrometry (MGA 1100, Perkin Elmer, Pomona, CA) until the CO_2 concentration exceeded 0.85%. Rats were then taken from the chamber and their weights and colonic temperatures (T_c) recorded immediately. Their locations in the chamber and order of removal for measurement were randomized to reduce systematic errors in temperature recording. T_c was measured at a depth of 6 cm using a YSI 402 probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Oxygen consumption ($\dot{V}\text{O}_2$) for the entire group was calculated from changes in O_2 volume while the chamber was hermetically sealed. As such, it represented a timed average over a 1–2 hour period and was expressed in milliliters (STPD) per hour per gram body weight.

The mass spectrometer was calibrated prior to each study. In addition, the chamber was calibrated weekly by measuring the $\dot{V}\text{O}_2$ of methanol combustion and comparing the measured rate with that predicted from the change in methanol weight. This measured $\dot{V}\text{O}_2$ was always within 2% of what was predicted. The temperature probe was calibrated in a stirred water bath after each study and the recorded values adjusted to ± 0.1 C accuracy.

Study Design

Prior to injury, studies were performed at the same time each day until $\dot{V}\text{O}_2$ and T_c reached minimal levels. Each animal was then anesthetized (sodium pentobarbital, 5 mg/100 g body weight, intraperitoneally), and the hair clipped from the back and flanks. The anesthetized rat was placed in a mold exposing 30% of the total body surface, and a full-thickness burn was produced over this area by immersing it in 98 C water for 9 seconds. Control animals were anesthetized and clipped but not burned.

Burn wounds of some groups were seeded immediately after injury. Seeding cultures contained 10^8 organisms per milliliter and one milliliter of culture medium was spread over the entire wound. One group was seeded with a virulent strain of *Pseudomonas aeruginosa* (ISR 59-12-4-4), four others with a nonvirulent *P. aeruginosa* (currently unclassified), and two more with *Staphylococcus epidermidis* (ATCC 12228). Some seeded burn wounds were treated once daily with Sulfamylon® (an 11.1% suspension of mafenide acetate in a water dispersible base) or Silvadene® (a 1% suspension of silver sulfadiazine in a water-miscible base). All treated animals were bathed weekly.

Multiple metabolic and temperature measurements were conducted between the sixth and 25th days after injury or sham burn. Animals were not studied if they presented clinical signs of sepsis, *i.e.*, markedly elevated T_c , excessive weight loss, weakness/lethargy, and light brown discharge around the eyes or nose. The rats were killed after the final experiment. Blood and spleen cultures and wound specimens were obtained from a representative number in each group to establish the incidence of bacterial wound invasion and systemic infection. Clinically bacteremic animals had positive blood and/or spleen cultures and histologic evidence of wound invasion. Only groups that demonstrated 90% homogeneity (bacteremic or nonbacteremic) are included in this report.

Data Analysis

Burn groups were separated into bacteremic and nonbacteremic categories. Paired t-tests were performed to determine the significance of changes between pre- and postburn $\dot{V}\text{O}_2$ and T_c for each category. An unpaired t-test was used to determine whether changes in $\dot{V}\text{O}_2$ after injury were different in the two categories. The effects of Sulfamylon on $\dot{V}\text{O}_2$ of nonbacteremic burn groups were determined by a nonlinear regression analysis for each group and an analysis of variance and covariance of data collected between the seventh and 21st postburn days.

Results

Two unburned and 13 burned groups were studied (Table 1). Sulfamylon and Silvadene treated groups did not become bacteremic, but three untreated groups did, two spontaneously and one seeded with virulent *P. aeruginosa*. Oxygen uptake of the burned animals increased after injury while that of the unburned controls tended to decrease slowly over the 3-week period of observation. Since $\dot{V}\text{O}_2$ is expressed per gram body weight, some of the difference in O_2 uptake after injury and sham burn is a reflection of the tendency for the uninjured animals to gain weight, while the burned animals all lost weight.

The magnitude and rate of rise in $\dot{V}\text{O}_2$ of the burned animals were greater in the bacteremic groups (Fig. 1). At an ambient temperature of 32 C, $\dot{V}\text{O}_2$ of the bacteremic animals rose steadily for 3 weeks, while there was little increase in O_2 uptake of the nonbacteremic groups until late in the second week. $\dot{V}\text{O}_2$ of the three bacteremic groups rose from 0.83 ± 0.01 to 1.20 ± 0.01 ml/hr/g (mean \pm S.E., $p < 0.001$, paired t-test) as compared with an increase from 0.81 ± 0.01 to 0.99 ± 0.02 ml/hr/g ($p < 0.001$, paired t-test) for nine nonbacteremic groups studied in the same 32 C environment (Table 1). The greater increase in $\dot{V}\text{O}_2$ of the bacteremic rats ($p < 0.001$, unpaired t-test) was accompanied by a rise in T_c (36.8 ± 0.1 to 37.7 ± 0.1 C, $p < 0.01$, paired t-test); there was no significant change in T_c in the nonbacteremic

TABLE 1. Group Characteristics before (B) and after (A) Burn Injury*

Group	Number† B/A	Weight (g) B/A	$\dot{V}O_2$ (ml/hr/g) B30/A30	T_c (degrees C) B30/A30
Unburned				
1	30/30	477/480	0.82/0.75	36.9/36.8
2	30/30	522/554	0.82/0.79	37.1/37.1
Burned, bacteremic			B30/A32	B30/A32
3	30/18	537/494	0.85/1.19	36.6/37.4
4	30/18	485/470	0.80/1.18	36.7/37.7
5 VP	30/13	552/466	0.83/1.22	37.1/37.9
Burned, nonbacteremic			B30/A32/A34	B30/A32/A34
6	30/24	521/494	0.82/0.99/0.90	37.3/37.6/38.0
7	30/26	512/490	0.80/1.03	37.0/37.9
8	27/25	547/544	0.77/0.99/0.92	37.0/37.2/37.6
9 NVP	30/29	477/461	0.84/1.04/1.04	37.1/37.4/37.6
10 NVP	30/29	503/486	0.84/1.07/1.02	37.1/37.1/37.9
11 NVP, Su	29/24	511/488	0.82/1.05/0.93	37.1/37.2/37.6
12 NVP, Su	30/30	535/516	0.80/0.97	36.8/37.5
13 VP, Su	30/30	552/538	0.83/0.88	37.1/37.2
14 SE, Su	30/30	540/510	0.79/1.01	36.8/37.5
15 SE, Ag	30/19	510/498	0.80/0.88	37.5/36.9

* (B)before values are averages of the last three studies prior to injury. (A)after values were obtained during the final study after injury. Studies of uninjured rats were conducted at 30C (B30/A30), while burned animals were studied at 32 and/or 34C (A32/A34). Final studies were performed between the 18th and 25th postburn day (PBD) except for groups 5 and 13–15, which were on PBD 8, 12, 16, and 15, respectively. Weight, $\dot{V}O_2$ and T_c are group means. Standard errors ranged from ± 3 to ± 11 g for weight, from ± 0.00 to ± 0.01 ml/hr/g for $\dot{V}O_2$ and from ± 0.0 to ± 0.2 C for T_c .

† Number of rats in each group.

VP = seeded with virulent *P. aeruginosa*.

NVP = seeded with nonvirulent *P. aeruginosa*.

Su = Sulfamylon treated.

SE = seeded with *S. epidermidis*.

Ag = Silvadene treated.

groups (37.1 ± 0.1 to 37.3 ± 0.1 C). Animals whose T_c rose above 38 C usually presented other clinical signs of sepsis and were removed from the study.

Bacteremic groups lost weight more quickly than did nonbacteremic groups (4.50 ± 3.12 vs. 1.14 ± 0.17 g/day, $p < 0.05$ unpaired t-test), but this was largely the result of group 5 where seeding the wounds with the virulent strain of *P. aeruginosa* resulted in an average weight loss of 10.75 g/day. Differences in weight loss between nonbacteremic and bacteremic groups did not account for the difference in $\dot{V}O_2$, however, since there was no significant difference in body weight between these two groups at the time of final study nor was there a significant correlation between the rate of weight loss and per cent increase in $\dot{V}O_2$ for either group.

Sometime in the second or third week postinjury, $\dot{V}O_2$ of the nonbacteremic burn groups could be reduced by increasing ambient temperature from 32 to 34 C. In the five groups studied at both temperatures between the 18th and 25th postburn days (groups 6, 8–11), $\dot{V}O_2$ dropped from 1.05 ± 0.03 to 0.98 ± 0.02 ml/hr/g ($p < 0.05$, paired t-test) following this two degree increase in ambient tem-

perature. Raising ambient temperature to 36 C increased $\dot{V}O_2$ in three of four of these groups. T_c followed changes in ambient temperature.

Metabolic effects were evident in the seeded groups before bacteria could be detected in the blood. This was best demonstrated when burn wounds of four groups of animals (groups 9–12) were seeded with the same nonvirulent strain of *P. aeruginosa*. Sulfamylon antimicrobial cream was applied daily to the wounds of animals in two of these groups (groups 11 and 12) while the other two groups were not treated. Treated and untreated groups remained nonbacteremic, but Sulfamylon treatment reduced the postburn rise in $\dot{V}O_2$ (Fig. 2). The effects of treatment were evident in both 32 and 34 C environments. The data were first examined by nonlinear regression analysis for each group, expressing $\dot{V}O_2$ (in ml/hr/g) as a function of postburn day (PBD). Mean $\dot{V}O_2$ was $1.02979 - 0.189489e^{-0.151106(PBD)}$ for the untreated groups and $0.928852 - 0.116472e^{-0.91194(PBD)}$ for the treated groups. There was no significant difference in mean $\dot{V}O_2$ of the treated groups between PBDs 7–21 (one-way analysis of variance and covariance), but in the untreated groups the mean $\dot{V}O_2$ of group 9 was greater than that of group 10 (1.02 vs. 0.97 , $p < 0.05$). Over these 2 weeks, mean $\dot{V}O_2$ of the untreated groups was significantly greater than that of the treated groups (0.99 vs. 0.92 , $p < 0.001$).

Sulfamylon treatment also prevented the lethal effects of the virulent strain of *P. aeruginosa*, but these animals (group 13) expressed a greater initial rise in O_2 than did

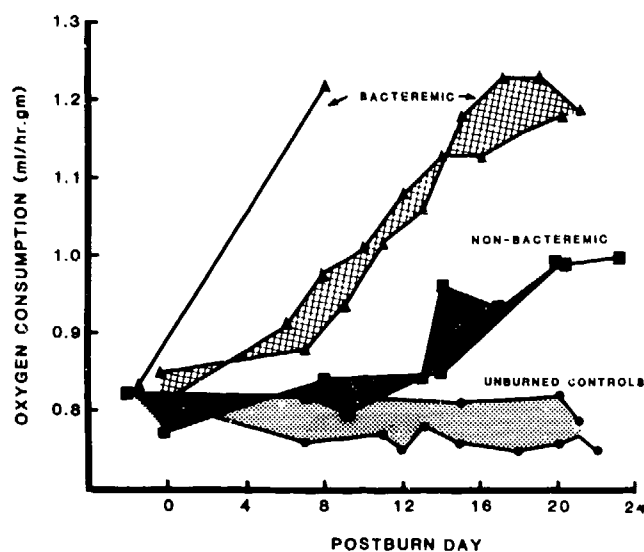


FIG. 1. Effects of different levels of infection on the oxygen consumption of groups of burned rats. The bacteremic group on the extreme left had wounds seeded with a virulent strain of *P. aeruginosa* while the other two groups became bacteremic spontaneously. Oxygen consumption values prior to zero postburn day represent group means of the last three studies before injury. Studies were conducted at an ambient temperature of 30 C before injury and 32 C after injury.

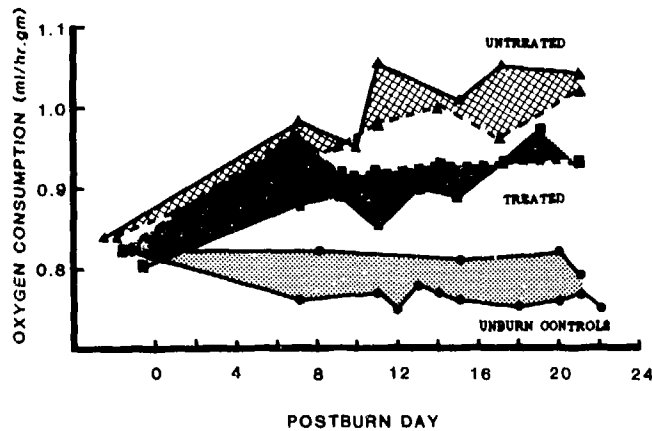


FIG. 2. Effects of Sulfamylon® treatment on the oxygen consumption of nonbacteremic rats whose wounds had been seeded with a nonvirulent strain of *P. aeruginosa*. Dashed lines are burned studies conducted at 32 C and solid lines are those at 34 C. Unburned controls were studied at 30 C. Oxygen consumption values prior to zero postburn day represent group means of the last three studies before injury.

animals infected with the nonvirulent mutant (Fig. 3). In addition, Sulfamylon appeared more effective in limiting the increase in $\dot{V}O_2$ with gram negative infection than it did with *S. epidermidis* infection. For example, it reduced the metabolic response to the virulent *P. aeruginosa* by the 11th, PBD, while the $\dot{V}O_2$ of treated rats infected with *S. epidermidis* continued to rise. Silvadene was more effective in reducing the metabolic cost of *S. epidermidis* infection (Fig. 4). All of these studies were conducted in a 32 C environment.

Discussion

These results indicate that bacteria and/or their products contribute to the rise in $\dot{V}O_2$ of the thermally injured rat. At an ambient temperature of 32 C, the rise in $\dot{V}O_2$ of burned animals varied with the severity of infection, rising 40 to 48% above normal in three bacteremic groups as compared to 21 and 28% in two untreated nonbacteremic groups (Fig. 1). The response to burn wound bacteria first appears as an elevation in $\dot{V}O_2$ without a measurable increase in T_{re} . As such, these nonbacteremic burn rats were like other animal models with comparable size burns.^{7,8} Bacteremic animals were febrile in the 32 C environment, but the nonbacteremic animals were not. The addition of a febrile drive with advancing infection may be responsible for a major portion of the increase in $\dot{V}O_2$ of the bacteremic animals.

Metabolic and febrile responses to invasive burn wound infection are well known, but there is a tendency to consider nonbacteremic patients as "free of infection."⁹ The present data indicate that such a concept may be misleading, since localized bacterial contamination of the

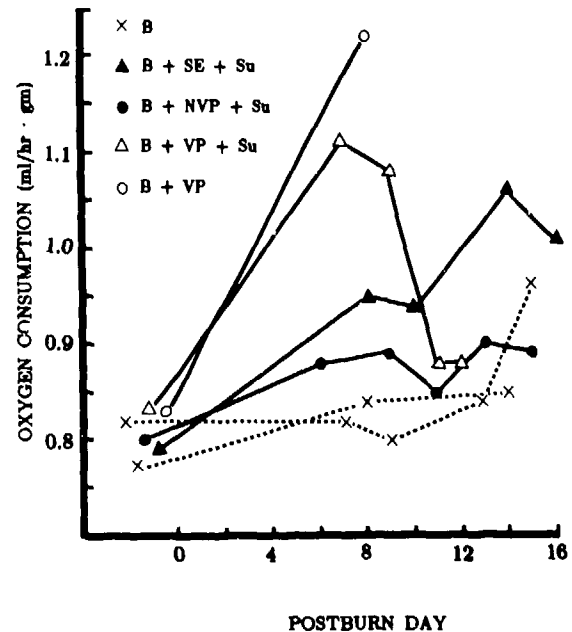


FIG. 3. Effects of Sulfamylon® treatment (Su) on oxygen consumption of nonbacteremic animals whose wounds were either unseeded (B) or seeded with different bacteria: virulent *P. aeruginosa* (VP), nonvirulent *P. aeruginosa* (NVP), or *S. epidermidis* (SE). Oxygen consumption values prior to zero postburn day represent group means of the last three studies before injury. Studies were conducted at an ambient temperature of 30 C before injury and 32 C after injury.

wound does have systemic metabolic consequences. By reducing $\dot{V}O_2$ with topical antimicrobial therapy, we have shown that bacteria were responsible for part of the increase in total body $\dot{V}O_2$ in nonbacteremic burned ani-

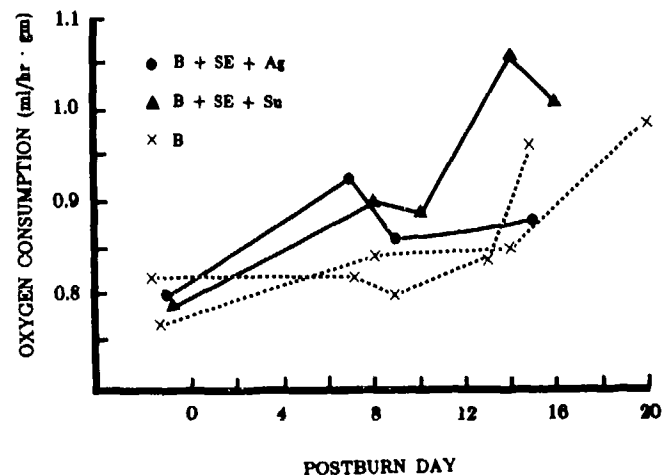


FIG. 4. Effects of different topical antimicrobial agents (Su = Sulfamylon®, Ag = Silvadene®, and X = no treatment) on the oxygen consumption of nonbacteremic rats whose wounds had been left unseeded (B) or seeded with *S. epidermidis* (SE). Oxygen consumption values prior to zero postburn day represent group means for the last three studies before injury. Studies were conducted at an ambient temperature of 30 C before injury and 32 C after injury.

mals. The energy cost of localized infection was not a function of differences in ambient temperature for it was evident at both 32 and 34 C (Fig. 2). Instead, it appeared to be determined by the bacterial strain involved, its virulence, and the effectiveness of the antimicrobial agent used to treat the wound (Figs. 3 and 4).

Sometime in the second or third week postinjury, $\dot{V}O_2$ of the nonbacteremic animals could be reduced by raising ambient temperature from 32 to 34 C. This shift in the thermal neutral zone is probably a result of increased evaporative heat loss from the wound following eschar separation.¹⁰ Once this occurs, $\dot{V}O_2$ of the burned rat (bacteremic or nonbacteremic) in the 32 C environment reflects the metabolic costs of injury/infection plus those imposed by the added cold drive. The decrease in $\dot{V}O_2$ upon moving into a warmer environment indicates that external heating rather than increased metabolic heat production was responsible for the associated increase in T_c .

Since bacteria are always present in the burn wounds of nonbacteremic, hypermetabolic patients, it is legitimate to ask whether burn injury itself has *any* measurable, direct effect on the $\dot{V}O_2$ of this model. While it is impossible to separate the metabolic effects of infection and injury completely, a rough estimate can be made by selecting nonbacteremic groups with limited changes in $\dot{V}O_2$ and assuming their infection component to be minimal or nonexistent. In two such groups, 6 and 11, $\dot{V}O_2$ at 34 C increased only 10 and 13% above preburn levels by the third week postinjury. In these two groups, T_c rose from 37.3 to 38.0 C and from 37.1 to 37.6 C, respectively. If these changes in core temperature are the sole result of external heating, how much of the observed change in $\dot{V}O_2$ could be explained by the Q10 effect of temperature on reaction rates? With the relationship established by DuBois,¹¹ a 13% increase in $\dot{V}O_2$ for every degree centigrade rise in core temperature, the hypermetabolism of these two particular groups might be largely a result of experimentally induced hyperthermia. In other words, after minimizing the infection component and correcting for the Q10 effect, there was little measurable "injury" hypermetabolism in this burn model.

Are those observations in the rat model consistent with clinical findings? The energy cost of localized wound bacterial growth is unknown for burned patients, but there is every reason to assume that the human response is as great as that of lower animals. Nonbacteremic patients, for example, are more hypermetabolic and febrile than animal models with the same size burn injury.^{7,8,10,12,13}

Humoral factors, which provide a link between wound microbes and total body energy metabolism, appear to play an important role in the afferent limb of the hypermetabolic response of burn patients.¹⁴ Endogenous pyrogen, one such factor related to bacterial contamination, has been identified in the sera of nonbacteremic burn patients.¹⁵ There was little evidence of pyrogens in the nonbacteremic burned rats, but the metabolic consequences of this family of endogenous mediators are numerous and frequently evident without changes in body temperature.¹⁶ For example, changes in plasma trace metals commonly attributed to the actions of leukocytic endogenous mediator have been found in the same non-bacteremic burn model used in this study (Aulick, Burleson, Mason, unpublished observations). The animal and human data taken together suggest that further study is warranted to assess the role of bacterial wound contamination in post-burn hypermetabolism of nonbacteremic patients.

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